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^{212}Bi-DOTMP: An Alpha Particle Emitting Bone-Seeking Agent for Targeted Radiotherapy

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ABSTRACT. The synthesis and in vivo stability of the bone-seeking α-particle emitting compounds ^{212}Bi-DOTMP and ^{212}Pb/^{212}Bi-DOTMP are described. ^{212}Bi-DOTMP, injected IV into Balb/c mice, showed prominent bone localization and a rapid clearance from blood and other organs. Femur/blood ratios increased from 13 at 15 min up to 490 at 2.0 h postinjection. Enhanced uptake of ^{212}Bi-DOTMP was demonstrated in regions with high bone turnover. A comparison between ^{212}Bi-DOTMP and [^{153}Sm]Sm-EDTMP showed essentially no differences in biodistribution. ^{212}Pb/^{212}Bi-DOTMP followed a similar biodistribution, except for slightly elevated levels of ^{212}Bi in the kidneys. The present study has shown ^{212}Bi-DOTMP to be an in vivo stable bone-seeking radiopharmaceutical with promising biological properties for the treatment of sclerotic metastases and osteoblastic osteosarcoma.   NUCL MED BIOL 24;3:231–237, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. ^{212}Bi-DOTMP, α-Emitter, Bone-seeking agent

INTRODUCTION

In recent years the focus on radiolabeled molecules as tumor therapeutics has increased (25). The reason for this interest is the high affinity and specific in vivo tumor targeting observed for several of these compounds. In particular, some smaller molecules (<1 kDa) have been shown to give high target to nontarget tissue ratios shortly after injection (1, 2, 17).

Metastases to the skeleton from prostate, breast, and lung cancer is a frequent and painful condition. The malignant cells induce pathological bone turnover by osteoclast activation, and in some cases pronounced bone formation by osteoblasts occurs at metastatic sites. Some bone-seeking radiopharmaceuticals accumulate rapidly and with strong enrichment after IV injection in regions with osteoblastic activity (19, 21). For instance, patients treated with [^{153}Sm]Sm-EDTMP, a tetraphosphonate labeled with a β-emitter, frequently experience pain relief (1, 5). In one clinical study, lesion/normal bone ratios of 4 were found for the diagnostic radiopharmaceutical ^{99m}Tc-HDP and for [^{153}Sm]Sm-EDTMP (6). The total skeletal uptake of [^{153}Sm]Sm-EDTMP varied from 40 to 90% of the injected dose (%ID) (6).

New bone formation is also a characteristic feature in a majority of osteosarcomas. In this case the tumor cells themselves produce osteoide, and primitive bone is deposited in the intercellular matrix. Both primary tumors as well as bone and soft tissue metastases produce primitive bone matrix, and "intense hot spots" are observed on conventional diagnostic bone scans (^{99m}Tc-MDP) (4, 26). The radioactivity contents in samples from osteosarcoma metastases and various normal tissues obtained at surgery 16 h after injection of ^{99m}Tc-MDP have been measured (4). Tumor/normal bone ratios of 5–10 and metastases/normal lung ratios as high as 100–300 were found. Hence, bone-seeking compounds are interesting candidates

for therapy of this type of tumor. In a study by Lattimer et al. (20), 40 dogs with bone tumors received [^{153}Sm]Sm-EDTMP and 80% responded positively to the treatment. Recently, Bruland et al. (3) have reported the successful use of [^{153}Sm]Sm-EDTMP as palliative treatment in a patient with inoperable relapsing osteosarcoma, and also reported a case with combined therapy in a dog with osteosarcoma (23).

In treatment employing bone-seeking radiopharmaceuticals labeled with β-emitters, the dose-limiting factor has always been bone marrow toxicity (1, 5, 14). By replacing β-particles with α-particles one might achieve better dose deposition on the target and less damage to the surrounding radiation-sensitive bone marrow. The α-particles from ^{212}Bi leave densely ionized tracks when traversing matter and have Linear Energy Transfer (LET) values close to the theoretical optimum of 100 keV/μm (11). Compared to the β-emitters, which have LET values of approximately 1 keV/μm, the difference in energy deposition per decay and cell is considerable. Because of this difference, α-particles are much more efficient for cell sterilization than β-particles. Furthermore, the dose-survival relationship of cells irradiated by α-particles is virtually independent of dose rate and oxygen content (11). Humm (15) has calculated the probability for cell sterilization after exposing cells with β-particles from ^{90}Y or with α-particles from ^{211}At. In a theoretical system with surface-bound radioactivity on single cells, he found that 99% cell death required 300 α-particles bound per cell in contrast to 361,000 β-particles per cell. In fact, even a single α-particle traversal of a cell nucleus has a high probability of killing the cell (7).

The ranges of the α-particles from ^{212}Bi are only 60–90 μm (a few cell diameters), considerably shorter than β-particles. Considering the proximity of the bone surface to the bone marrow cells, a further reduction in hematological toxicity may be accomplished by changing to α-particle emitters.

The α-particle emitter ^{212}Bi is the daughter nuclide of ^{212}Pb, which is readily available from a ^{228}Th generator (12). The physical half-life of ^{212}Bi is only 1.0 h. Thus, if this radionuclide is to be used
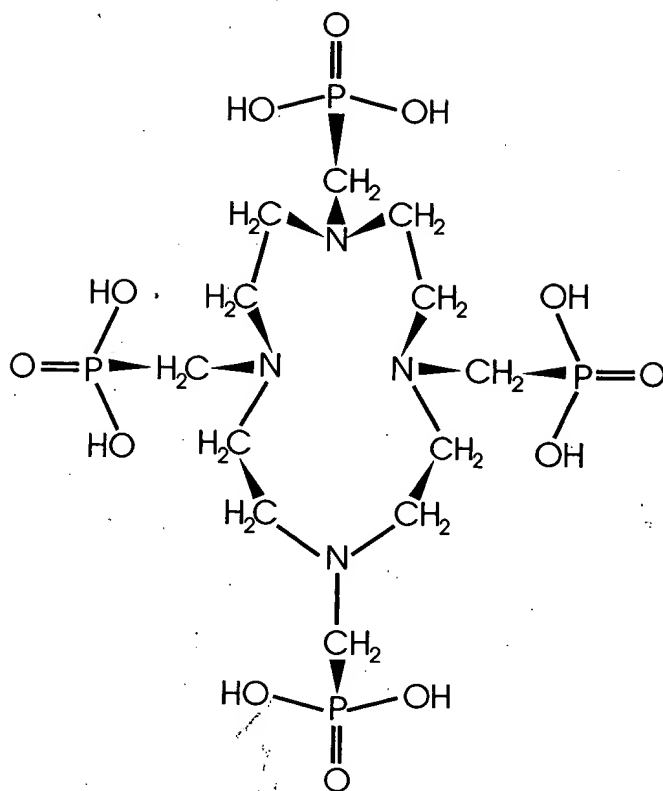
FIG. 1. Structure of DOTMP (1,4,7,10-tetraazacyclodode-cane-1,4,7,10-tetra(methylene-phosphonic acid)).

directly, both rapid tumor enrichment and clearance from blood and nontarget organs are imperative. Because a 1.0-h half-life may be too short, the use of the precursor ^{212}Pb ($t_{1/2} = 10.6$ h), a pure low-energy β^--emitter might be a possible alternative.

Biodistribution studies of [^{153}Sm]Sm-EDTMP in rats showed that skeletal localization and clearance from blood and organs are achieved within minutes following injection (8). Studies in patients given [^{153}Sm]Sm-EDTMP for palliative treatment of bone metastases showed that the radiopharmaceutical was predominantly cleared from blood by an initial 5.5-min half-life during the first 30 min (1). It therefore seems possible that sclerotic bone metastases and osteoblastic osteosarcoma are suitable candidates for α-particle targeted radiotherapy with ^{212}Bi, provided that the radionuclide can be stably attached to a bone-seeking vehicle. In a previous paper we investigated the biodistribution of ^{212}Pb/^{212}Bi-EDTMP, which showed good bone localization but with relatively high kidney values of ^{212}Bi (13). Because this biodistribution study showed ^{212}Pb/^{212}Bi-EDTMP to have rapid kinetics, *in vivo* stability of ^{212}Bi-EDTMP was investigated, but EDTMP was found to be an unsatisfactory chelator for ^{212}Bi (unpublished data). Thus, in search for an *in vivo* stable chelator for ^{212}Bi, we have in this paper explored the usefulness of DOTMP.

MATERIALS AND METHODS
Synthesis of DOTMP and EDTMP

^{1}H-NMR (D$_2$O) EDTMP: 2.73 ppm (singlets, NCH$_2$CH$_2$N), 2.50, 2.56 ppm (dublets, NCH$_2$P)

DOTMP: 2.71 ppm (singlets, NCH$_2$CH$_2$N), 2.63, 2.69 ppm (dublets, NCH$_2$P)

^{31}P-NMR (D$_2$O) EDTMP: 17.6–17.9 ppm (triplets, CH$_2$PO$_3$)

DOTMP: 18.5–18.8 ppm (triplets, CH$_2$PO$_3$)

The tetraphosphonates DOTMP (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylene-phosphonic acid)) (Fig. 1) and EDTMP (ethylene-diamine-tetra(methylene-phosphonic acid)) were synthesised according to a Mannich-type reaction as outlined by Moedritzer and Irani (24). For the synthesis of DOTMP, described by Simon et al. (29), 250 mg of 1,4,7,10-tetraazacyclododecane and 492 mg of phosphorous acid were dissolved in 5 mL of 4 M HCl and heated to reflux temperature (~105–110°C). Thereafter 1.0 mL (100% excess) of a 37% aqueous [^{14}C]formaldehyde (NEN Research Products) solution was added in small amounts by a 1.0-mL syringe over the course of 1 h. After refluxing for an additional 2 h, the reaction mixture was allowed to cool to room temperature, then evaporized on a rotavapor to a reddish-brown oil-like substance, dissolved in distilled water and subsequently precipitated by adding acetone. This procedure was repeated three times, and the final precipitate was dissolved in 2.5 mL distilled water and pH adjusted to 0 by adding HCl. Seed crystals were added and the solution was stored at ~4°C. After a few days white crystals had formed at the bottom of the beaker. The crystalized DOTMP was further purified by one recrystallization in 1 M HCl, and dried in a desiccator for 2 days. The final product had a specific ^{14}C activity of 0.25 GBq/mol (6.7 mCi/mol), determined by liquid scintillation counting (Beckman LS 6500).

The synthesis of EDTMP followed the reaction sequence outlined for DOTMP but with ethylenediamine instead of 1,4,7,10-tetraazacyclododecane and ordinary formaldehyde instead of [^{14}C]formaldehyde. EDTMP crystallized directly out of the reaction mixture, and was purified by one recrystallization in 1 M HCl.

All nonradioactive chemicals were purchased from Fluka Chemical Co. and NMR spectra (^{1}H and ^{31}P) were obtained in D$_2$O, NaOD using a Varian 200 MHz instrument.

Preparation of Radioconjugates

Both ^{212}Pb and ^{212}Bi were obtained from a radionuclide generator (12) based on the principle of collecting gaseous ^{220}Rn emanating from barium stearate doped with ^{228}Th. The ^{220}Rn ($t_{1/2} = 55.6$ sec) is trapped in a 250-mL polyethylene bottle, where it decays to ^{212}Pb ($t_{1/2} = 10.6$ h). The ^{212}Pb deposits on the walls of the bottle and can be easily washed off with an appropriate solution. As ^{212}Pb is the precursor of the α-particle emitter ^{212}Bi ($t_{1/2} = 60.6$ min), the solution will contain both radionuclides.

^{212}Bi-DOTMP. To obtain pure ^{212}Bi-DOTMP, the polyethylene bottle was shaken with 5 mL of 0.1 M HNO$_3$ for 5 min. By this method ^{212}Pb/^{212}Bi were washed off to more than 90% yield. This solution was eluted through a 2- × 10-mm column, containing the strong cation exchange resin AG 50W-X4 (Bio-Rad), retarding ^{212}Pb quantitatively. The column was then washed with 0.2 mL of 0.1 M HNO$_3$, and allowed to stand for more than 2 h to ensure that ^{212}Bi was in equilibrium with ^{212}Pb. Approximately 80% of the ^{212}Bi, with <0.15% ^{212}Pb contamination, was then eluted with 0.10 mL of 0.10 M HI, and added to a solution of 0.20 M DOTMP. The pH of the reaction solution was now 7, and the complexation reaction was allowed to proceed for 45 min at approximately 70°C. After dilution of the reaction solution with distilled water and addition of sodium phosphate saline buffer to physiological conditions, the final solution was 7 mM in DOTMP and 2 MBq/mL (0.05 mCi/mL) in ^{212}Bi. It is possible that the complexation kinetics of bismuth to the chelator may be slow. This has been reported for

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylene-acetic acid)) (16), which has the same structural backbone as DOTMP. Thus, to ensure complete chelation of ^{212}Bi to DOTMP, long reaction time, high reaction temperature, and high DOTMP concentration were applied. The biodistribution data (e.g., low kidney values) confirm the complete chelation of ^{212}Bi to DOTMP. A chromatographic system for separation of uncomplexed ^{212}Bi from ^{212}Bi-DOTMP could not be developed, owing to the complicated aqueous chemistry of bismuth.

$^{212}Pb/^{212}Bi$-DOTMP. For chelation of the mixture of ^{212}Pb and ^{212}Bi to DOTMP, the polyethylene bottle was washed with 30 mL of distilled water containing the desired amount of DOTMP. After 5 min of shaking, ~90% of the ^{212}Pb activity was washed off the walls. This solution was then concentrated on a rotavapor to approximately 1 mL and added 1 M NaOH to pH ≈ 8. After 15 min reaction time at ~70°C, the solution was eluted through a 2- × 10-mm Chelex 100 column to remove unbound ^{212}Pb. After adjusting to physiological conditions by adding sodium phosphate saline buffer, the solution was 10 mM in DOTMP and 4 MBq/mL (0.1 mCi/mL) in both ^{212}Pb and ^{212}Bi.

$[^{153}Sm]Sm$-EDTMP. The $[^{153}Sm]Sm$ was produced through neutron irradiation of natural Sm_2O_3 by Institute for Energy (IFE) at Kjeller, Norway, using a thermal flux of $1 × 10^{13}$ neutrons/cm² s¹ for 2 days. $[^{153}Sm]Sm$-EDTMP was prepared by dissolving the neutron-activated natural Sm_2O_3 in 0.1 M HCl and adding the desired amount of radioactivity to the EDTMP solution. After adjusting the pH to 9 with 1 M NaOH, a reaction time of 15 min was applied. The solution was then eluted through a 2-×-10-mm Chelex 100 column for removal of unbound $[^{153}Sm]Sm^{3+}$ and then added sodium phosphate saline buffer to physiological conditions. The final solution was 1.2 mM in Sm; 20 MBq ^{153}Sm/mL (0.54 mCi/ml) and 13 mM in EDTMP.

Before injection, all solutions were filtered through a 0.2-μ sterile syringe filter (Nalgene).

Biodistribution Experiments

Unanesthetized Balb/c female mice had injected into the tail vein 0.10 mL of either ^{212}Bi-DOTMP, $^{212}Pb/^{212}Bi$-DOTMP, or the $[^{153}Sm]Sm$-EDTMP solution. The distribution of ^{212}Bi-DOTMP were performed on three different age groups, weighing 15–16 g, 18–19 g, and 25–26 g. The mice receiving $^{212}Pb/^{212}Bi$-DOTMP weighed 18–19 g, while $[^{153}Sm]Sm$-EDTMP was injected into mice weighing 15–16 g. The mice were killed by cervical dislocation, and blood samples from the heart/thoracic region were immediately obtained, followed by removal of the various organs. Two different bone specimens were dissected, i.e., the femur containing "growth zones" and the calvarium from the skull bone representing "flat bone." All samples were weighed and the radioactivity content measured. Results are expressed in terms of % of injected dose per gram (%ID/g). The organ ratios are calculated by dividing the %ID/g for femur with the %ID/g for the actual organ. All animal experiments were performed in accordance with national regulations.

Radioactivity Measurements

To discriminate between ^{212}Pb and ^{212}Bi in the samples containing both radionuclides, measurements were performed on a calibrated 50% high-purity Ge γ-ray detector (Canberra) coupled to a multichannel analyzer (EG & Ortec). The ^{212}Pb activity was determined by measurement of its 238.6 keV (43.6%) γ-ray, and to quantify the ^{212}Bi content, the 583.1 keV (32.5%) γ-ray from ^{208}Tl at transient equilibrium with the parent was measured. Samples containing pure ^{212}Bi were counted on a NaI(Tl) scintillation detector coupled to a scaler/timer unit, whereas the activities from ^{153}Sm were determined by measurements of its 103 keV γ-ray (78%) on a calibrated low-energy Ge detector (Canberra) coupled to a multichannel analyzer (EG & Ortec). All radioactivity measurements of the samples were compared to diluted standards, and thereby automatically corrected for decay and differences in detection efficiency.

Organs containing DOTMP were prepared for ^{14}C counting by the following procedure: Samples of whole blood (0.1–0.4 mL) were mixed in the counting vial with 1.0 mL isopropanol/SOLUENE-350 1:1 mixture, and then 0.5 mL of 35% hydrogen peroxide was added dropwise under gentle swirling. After 30 min at 40°C, 15 mL of 0.5 M of HCl/INSTA-GEL 1:9 mixture was added to each vial. The bone samples (50–100 mg) were dissolved during 1 h at 80°C in a mixture containing 0.2 mL of 60% perchloric acid and 0.4 mL of 35% hydrogen peroxide. After cooling, 15 mL of INSTA-GEL was added to all vials. The other organs were dissolved by addition of 1.0 mL of SOLUENE-350 per 100 mg tissue, heated for 4 h at 50°C, and then given 15 mL of INSTA-GEL. To avoid chemiluminescence, all vials were allowed to stand for 1 day before counting. Measurements of ^{14}C in blood and the different organs are a direct measure of the DOTMP content. SOLUENE-350 and INSTA-GEL were purchased from Packard Chemical Co., and the ^{14}C measurements were performed on a Beckman LS 6500 multi-purpose scintillation counter.

RESULTS
Biodistribution of ^{212}Bi-DOTMP and $^{212}Pb/^{212}Bi$-DOTMP

^{212}Bi-DOTMP. The biodistribution data show that ^{212}Bi-DOTMP is rapidly taken up in the bone matrix with a fast clearance from blood and other organs. The maximum uptake of ^{212}Bi of 26% ID/g was reached in the femur 15 min postinjection (Fig. 2). At this time point the femur/blood ratio was 13, rapidly increasing to 190 and 490 at the 1.0 and 2.0 h time points, respectively. The rapid excretion is illustrated in Fig. 3 by comparing the ^{212}Bi contents in femur, blood, and lung. A comparison of the biodistribution of ^{212}Bi-DOTMP with the similar biodistribution study of $[^{153}Sm]Sm$-EDTMP revealed no differences in biodistribution pattern. This is clearly seen in Fig. 4, which shows the distribution at the 2.0 h time point for both radiolabeled phosphonates. The ^{14}C measurements confirm the analogous biodistribution of the polyphosphonate DOTMP, and the radionuclide ^{212}Bi, in every organ studied and at all time points (Fig. 2). Urine was collected from the three animals at the 1.0 h time point, which spontaneously emptied their bladder at the moment of death. Also, the contents of ^{212}Bi and ^{14}C here were the same. The ^{212}Bi values were 40, 4.0, and 33% ID, compared to ^{14}C measurements of 39, 3.5, and 32% ID.

The ^{212}Bi-DOTMP contents in the femur were lower in older animals compared to younger ones. The animals weighing 18–19 g had values in the range of 14% ID/g, and those weighing 25–26 g had approximately 8% ID/g. The uptake of ^{212}Bi-DOTMP in the skull bone showed much less age variation compared to the uptake of ^{212}Bi-DOTMP in the femur. The youngest mice, weighing 15–16 g, had about 11% ID/g, whereas mice weighing 18–19 g had values on the order of 9% ID/g, and the oldest, weighing 25–26 g, approximately 6% ID/g. The clearance of ^{212}Bi-DOTMP from blood and organs in the older animals was no different from the younger
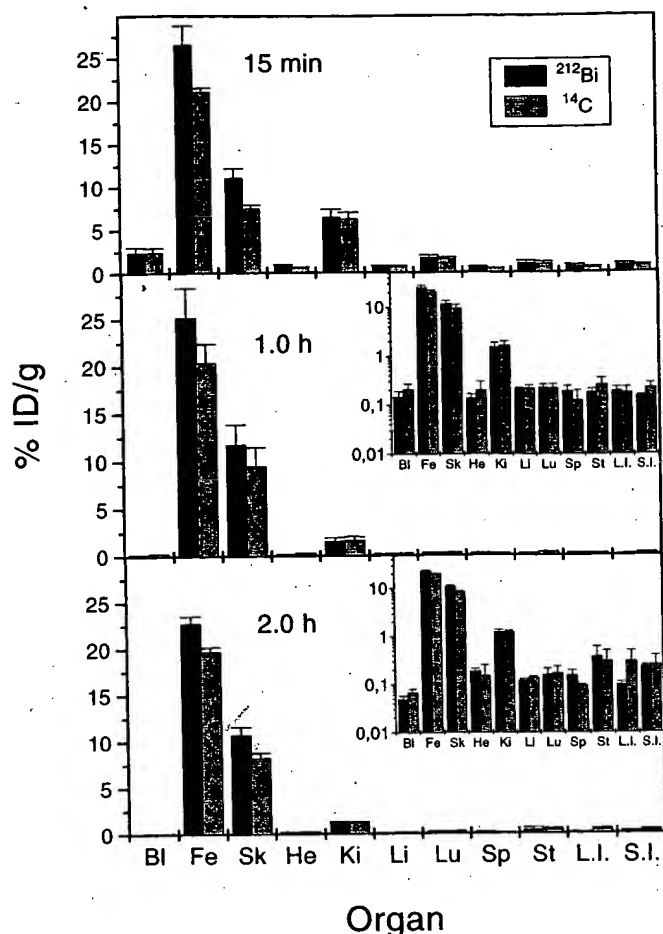
FIG. 2. Biodistribution of IV-administered ^{212}Bi-DOTMP in Balb/c mice (15–16 g). The amounts of ^{212}Bi and of ^{14}C-labeled DOTMP are plotted as %ID/g for several organs at 15 min, 1.0 h, and 2.0 h postinjection. Each animal received 0.10 mL of a solution 7.0 mM in DOTMP and 2 MBq ^{212}Bi/mL (0.05 mCi/mL). Values are averages ± SE of three mice. (Bl = blood, Fe = femur, Sk = skull, He = heart, Ki = kidney, Li = liver, Lu = lung, Sp = spleen, St = stomach, L.I. = large intestine, and S.I. = small intestine).
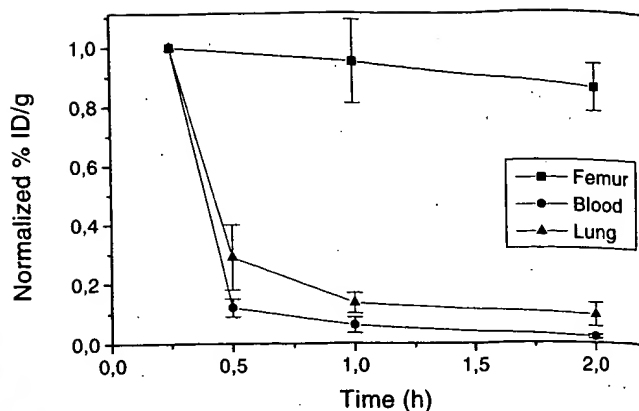

FIG. 3. The clearance of ^{212}Bi-DOTMP in Balb/c mice (15–16 g). The %ID/g values of ^{212}Bi for femur, blood, and lung are normalized to 1 at the 15 min time point. Values are averages ± SE of three mice. (Animals at the 0.5 h time point weighed 18–19 g.)

levels coincided (16% ID/g and 18% ID/g, respectively), and were somewhat higher than the ^{212}Pb level (10% ID/g). The ^{212}Pb and ^{14}C levels showed little change, but the ^{212}Bi content dropped to a stable level of 6% ID/g at the two later time points.

DISCUSSION

The aim of the present work was to produce an *in vivo* stable bone-seeking ^{212}Bi-labeled compound with rapid kinetics following IV injection.

^{212}Bi-DOTMP

The biodistribution data of the radiolabeled polyphosphonate ^{212}Bi-DOTMP showed prominent enrichment in normal bone matrix and a rapid clearance from blood and other organs. In vivo stability of ^{212}Bi-DOTMP was evident from the fact that identical biodistribution patterns between ^{212}Bi and ^{14}C-labeled DOTMP were found. Furthermore, these results show that ^{212}Bi is deposited in the bone as intact ^{212}Bi-DOTMP complex. The high stability of ^{212}Bi-DOTMP was further corroborated by the low kidney values. Several groups have shown that approximately 50% of injected uncomplexed bismuth (III) is retained by the kidneys (10, 28, 30). Hence, if the compound had been unstable, very high kidney values and lower bone values would have been observed.

To assess better the bone-seeking ability and clearance, ^{212}Bi-DOTMP was compared with ^{153}Sm[Sm]-EDTMP in a biodistribution study in mice of the same age. We found essentially no differences in biodistribution patterns between these two compounds. Several radiolabeled poly- and bis-phosphonates have been studied in rats (8), and ^{153}Sm[Sm]-EDTMP has been shown to be most favorable when comparing bone-seeking ability and clearance from blood and nontarget organs. It is also reported that [^{153}Sm]Sm was complexed to EDTMP in urine samples (9), demonstrating *in vivo* stability of [^{153}Sm]Sm-EDTMP. In the present study we found equal levels of ^{212}Bi and ^{14}C-labeled DOTMP in urine samples, indicating stability. Measurements of stomach and intestine with and without contents have shown that ^{212}Bi-DOTMP to some extent is excreted in the contents of these organs.

As expected, ^{212}Bi-DOTMP levels in the femur were considerably higher than in skull bone owing to the presence of growth

ones, but followed the same rapid pattern. The ratios of femur/organ for the youngest and the oldest mice at the 1.0 h time point are presented in Table 1.

^{212}Pb/^{212}Bi-DOTMP. The biodistribution pattern of the tetraphosphonate DOTMP complexed with the mixture of the two radionuclides ^{212}Pb and ^{212}Bi was quite similar to ^{212}Bi-DOTMP. The enrichment of both radionuclides in the bone matrix was fast, together with a rapid clearance from blood and other organs, with the uptake of ^{212}Bi in the kidneys as the only exception (Fig. 5). The blood levels were 0.6% ID/g at the 30 min time point for both ^{212}Bi and ^{212}Pb, and decreased to 0.06% ID/g for ^{212}Bi and approximately 0.02% ID/g for ^{212}Pb at the later time points. Even if the kidney values were high, the ratio of femur/kidney always exceeds 1, with values in the range 1.4–2.8.

The ^{14}C measurements coincide with the measurements of both ^{212}Pb and ^{212}Bi except for kidneys and bone. In the kidneys there were elevated levels of ^{212}Bi compared to ^{212}Pb and ^{14}C at all time points. In the femur at the 30 min time point, the ^{212}Bi and ^{14}C
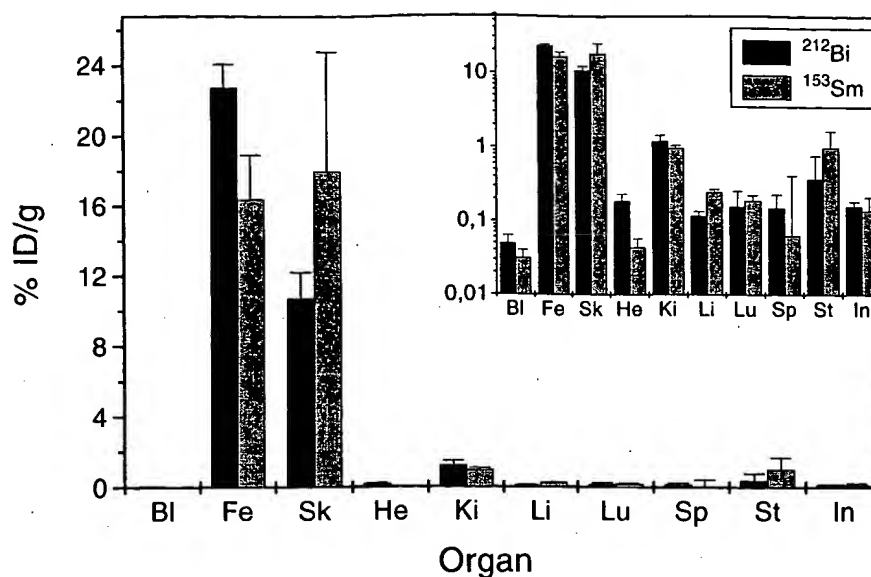
FIG. 4. A comparison between the biodistribution pattern of IV-administered ²¹²Bi-DOTMP or [¹⁵³Sm]Sm-EDTMP in Balb/c mice (15–16 g) 2.0 h after injection. The animals received 0.10 mL of a solution that was either 7.0 mM in DOTMP and 2 MBq (0.05 mCi/mL) ²¹²Bi/mL, or 13 mM EDTMP, 1.2 mM Sm^{3+} and 20 MBq ¹⁵³Sm/mL (0.54 mCi/mL). Values are averages ± SE of three mice. (Bl = blood, Fe = femur, Sk = skull, He = heart, Ki = kidney, Li = liver, Lu = lung, Sp = spleen, St = stomach, and In = intestine.)

zones with high bone turnover in the femur. To investigate this contribution further, bone values from older and younger animals were compared. Clear differences between the different age groups were found. The weight difference between the youngest and the oldest group was about 60%, whereas the radioactivity content was three times as high in femurs from the youngest group. The radioactivity levels in skull bone varied no more than expected from the weight difference between the three age groups. This clearly demonstrated that ²¹²Bi-DOTMP targets to a greater extent to regions with high bone turnover, such as the growth zones. The femur is a mixture of active and quiescent regions. The uptake of ²¹²Bi-DOTMP in osteoblastic ostesarcoma or sclerotic bone metastases might therefore be considerably higher than any bone-value found in this study.

TABLE 1. Ratio of Femur/Organ of ²¹²Bi-DOTMP in Young and Old Female Mice

Organ	Young mice	Old mice
Blood	190 (30)	73 (12)
Skull	2.19 (0.13)	1.27 (0.05)
Heart	190 (15)	100 (20)
Kidney	17 (2)	4.9 (0.5)
Liver	120 (10)	75 (2)
Lung	112 (6)	59 (6)
Spleen	170 (60)	74 (9)
Stomach	142 (9)	50 (20)
Large intestine	142 (7)	62 (14)
Small intestine	180 (40)	27 (10)

The table shows the ratio of femur/organ of ²¹²Bi in Balb/c female mice from two different age groups. The young mice weighed 15–16 g, and the old mice weighed 25–26 g. The ratio is calculated by dividing the %ID/g for femur with %ID/g for the specified organ. Each animal received 0.10 mL of a solution 7.0 mM in DOTMP and 2 MBq (0.05 mCi/mL) ²¹²Bi/mL. Values are averages of three mice, with SE in parentheses.

²¹²Pb/²¹²Bi-DOTMP

The intriguing possibility of in vivo generation of the α-particle emitter for therapy of osteosarcoma and bone metastases was investigated in a previous work with a biodistribution study of ²¹²Pb/²¹²Bi-EDTMP (13). Compared to that compound, ²¹²Pb/²¹²Bi-DOTMP has better in vivo stability. This is evident from the lower values of ²¹²Pb and ²¹²Bi in blood and organs, leading to considerably improved bone/blood and bone/organ ratios. Thus, this study has shown DOTMP to be a better chelator than EDTMP for both lead and bismuth.

In general, ²¹²Pb/²¹²Bi-DOTMP showed rapid bone localization and fast clearance from blood and other organs with the exception of elevated levels of ²¹²Bi in the kidneys compared to ²¹²Pb and DOTMP. This is not unexpected since Mirzadeh et al. (22) have shown that 36% of ²¹²Bi-DOTA formed from β⁻ decay of ²¹²Pb-DOTA is dissociated to free bismuth as a consequence of the nuclear transformation. The ²¹²Bi lost from the bone is efficiently trapped in the kidneys, as expected from the biodistribution of uncomplexed bismuth (10).

Despite this problem, injection of pure ²¹²Pb-DOTMP may be an alternative strategy. Large amounts of the injected radiolabeled polyphosphonate are excreted in the first minutes. By this, the initial high dose to bone marrow and kidneys from the α-particles might be reduced. However, after approximately 3 h the distribution of ²¹²Pb-DOTMP will be virtually the same as for an equilibrium mixture of ²¹²Pb and ²¹²Bi complexed with DOTMP. In a recent work of Ruble et al. (27), a ²¹²Pb-labeled monoclonal antibody was used in the treatment of a murine erythroleukemia. Elevated levels of ²¹²Bi in the kidneys did not seem to give any observable toxicity. On the other hand, bone marrow toxicity was a serious problem in this model. In another study by Huneke et al. (16), using the same tumor model and the same antibody but labeled with ²¹²Bi, bone marrow toxicity was not a problem. It therefore seems likely that ²¹²Bi lost from the chelator directly
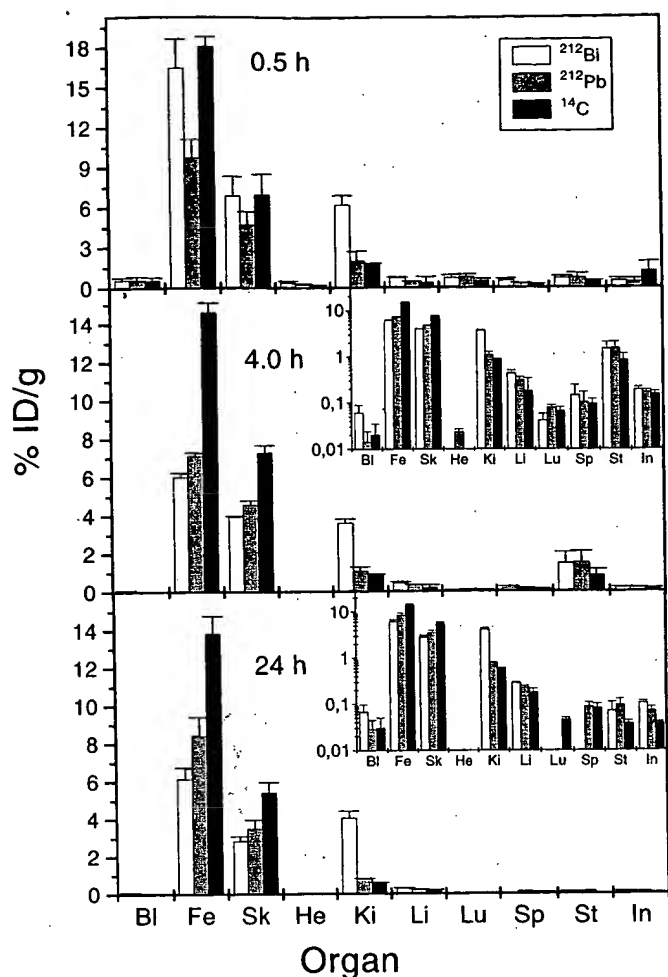
FIG. 5. Biodistribution of IV-administered ^{212}Pb/^{212}Bi-DOTMP in Balb/c mice (18–19 g). The amounts of ^{212}Pb, ^{212}Bi, and ^{14}C-labeled DOTMP are plotted as %ID/g for several organs at 0.5 h, 4.0 h, and 24 h postinjection. Each animal received 0.10 mL of a solution 10 mM in DOTMP and 4 MBq/mL (0.1 mCi/mL) in both ^{212}Pb and ^{212}Bi. Values are averages ± SE of three mice. Blank columns represent radioactivity levels below detection limit. (Bl = blood, Fe = femur, Sk = skull, He = heart, Ki = kidney, Li = liver, Lu = lung, Sp = spleen, St = stomach, and In = intestine.)

causes bone marrow toxicity. This may limit the use of ^{212}Pb-DOTMP.

In the present study the amounts of ^{212}Pb and ^{212}Bi in the bone matrix were quite similar at the 4.0 h and 24 h time points, indicating stable binding of ^{212}Pb and stable binding of the amount of ^{212}Bi not lost from the bone in the nuclear transformation. The difference between ^{212}Pb and ^{212}Bi in the bone specimens at the two later time points indicates that 70–85% of the *in vivo* produced ^{212}Bi is retained.

The close similarity between ^{14}C-labeled DOTMP and ^{212}Bi-DOTMP in the bone matrix (Fig. 2) is not as clear when comparing ^{14}C-labeled DOTMP and ^{212}Pb-DOTMP (Fig. 5). The slightly lower bone values of ^{212}Pb-DOTMP may be due to lower bone affinity of the compound or a chemical break-up of the complex. However, a chemical break-up is unlikely since the kidney and liver values then would have increased considerably more (10, 18). Furthermore, there is close resemblance between the radioactivity

content of ^{14}C-labeled DOTMP and ^{212}Pb-DOTMP in the other organs. It therefore seems likely that Pb-DOTMP has a slightly lower bone affinity compared to DOTMP and Bi-DOTMP.

In conclusion, we have shown the α-emitting tetraphosphonate ^{212}Bi-DOTMP to be an *in vivo*, stable bone-seeking compound. It is shown to rapidly enrich in regions with high bone turnover, making it a possible candidate for the treatment of sclerotic bone metastases and osteoblastic osteosarcoma. Because of the high LET and the short range of the α-particles, knowledge of the microdistribution of Bi-DOTMP in normal bone and in tumor regions is essential.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; CHAVEZ, MEDARDO R. ET AL: "New route to macrocyclic-based phosphonate acetoxymethyl (AM)-esters: synthesis, cell loading, and 31P NMR" retrieved from STN Database accession no. 132:191345 XP002170878 abstract & PROC. SPIE-INT. SOC. OPT. ENG. (1999), 3600(BIOMEDICAL IMAGING: REPORTERS, DYES, AND INSTRUMENTATION), 99-106 , ---	1-3,5, 7-11,13
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